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Anaerobic digestion of solid organic substrates in batch mode: An overview relating to methane yields and experimental procedures

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ABSTRACT

Anaerobic digestion is considered a competitive source for the production of renewable energy as far as efficiency and cost are concerned. To evaluate the anaerobic biodegradability of an organic substrate such as feedstocks, a test known as biochemical methane potential (BMP) has been commonly used. Current worldwide interest in using different organic substrates for anaerobic bioconversion is growing but there is a lack of clear references and comparability as a result of multiple factors that affect BMP determination. Several batch methods have been used to determine the methane potential. However, these technical approaches vary significantly from one reported method to the next another. In this review, the research works on the influence of different parameters of BMP determination have been discussed for critical and comparative evaluation. In addition, the extensive literature previously published dealing with BMP assays has been compiled and summarized focusing on two main subjects: firstly, methane yields of substrates, and secondly, the description of the various experimental procedures used to achieve the reported data.

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Contents

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1. Introduction

Anaerobic digestion (AD) is a biochemical technological process for the treatment of organic substrates such as sewage and industrial effluents, animal manures and solid substrates (energy crops, agricultural residues and food wastes). This process has received increasing attention in recent years. It involves the degradation and stabilisation of complex organic matter by a consortium of microorganisms leading to an energy-rich biogas which can be used as renewable energy to replace fossil energy sources.

Literature shows that anaerobic digestion assays can be carried out in batch or continuous mode. Considering that continuous set-up is more laborious and time-consuming than batch tests, the latter have been more widely used. It is important to note that the batch approach can be used for three purposes: anaerobic biodegradability, inoculum activity and inhibition. These terms were defined with the aim of establishing a common terminology [1]. These three tests are based on the same principle – the measurement of biogas/methane production. However, the protocols available in the literature differ not only with regard to the method used to quantify the gas produced during the test, but also with regard to the experimental conditions adopted for incubating the inoculum. Extensive research has been carried out to study the influence of experimental conditions on the results for inoculum activity and inhibition assays. On the other hand, studies on biodegradability, of which there have been much fewer, can be placed into two main groups following the nature of the substrate:

(i) Micro-pollutants (chemical compounds and plastics). Test methods for assessing anaerobic biodegradability of chemical substances have been previously described. Some of them studied the influence of key parameters such as compound and inoculum concentrations and mineral medium composition [2–5]. Moreover, there are standards and guidelines for anaerobic testing, reviewed by Müller et al. [6].
(ii) Complex organic substrates (manures, wastewaters, sludges, solid wastes). The first report of anaerobic biodegradability assessment in batch mode was carried out by Owen et al. [7]. This test was developed to determine the biochemical methane potential (BMP). There is less research available on the influence of key parameters in BMP of organic materials.

This review will focus on the AD of solid organic substrates (SOS). Reviews have been previously published which include data on AD experiments using solid substrates in batch and continuous mode [8–11]. In spite of the reviews published, the variety of methods reported in the literature for determining BMP and the discrepancies in approaches and results obtained for each experimental procedure emphasizes the need for an extended review. The purpose of this review article is to integrate all of the anaerobic biodegradability tests in batch mode for different solid substrates which have been previously reported in the literature. The aim of this review will be threefold: firstly, the text includes extensive information about the influence of different factors affecting the BMP results, secondly, the manuscript summarizes the important energetic data of methane potential (Appendix A) and thirdly, the document gives a detailed report of the different experimental procedures used in each case described (Appendix B).

2. Factors affecting the performance of anaerobic batch tests

The general principle of all batch tests is the incubation of an inoculum containing a variety of anaerobic microorganisms in a suitable medium (water and minerals) at neutral pH and at specific temperature range (normally mesophilic or thermophilic). Substrate is added to the medium and serves as a source of carbon and energy for the microorganisms. After incubation, the degree of degradation of the substrate is assessed at pre-set time intervals to determine its extent and conversion rate. Blank controls (endogenous tests, with the inoculum alone added) are included so that the gas produced from the organic matter contained in the inoculum can be accounted for.

Certain factors have the potential to affect the biodegradability assays and, therefore, the biogas/methane production. They are detailed in the following paragraphs:

2.1. Solid organic substrates (SOS)

Raw materials can be obtained from a variety of sources. Different groups of potential sources for methane production were considered by Gunaseelan [8] such as the organic fraction of municipal solid waste (OFMSW), fruit and vegetable waste (FVW), grasses, woods, terrestrial weeds, and aquatic (marine and freshwater) biomass.

2.1.1. Characterisation

It is known that the anaerobic biodegradability of organic matter is related to its composition [12–20]. Therefore, in order to carry out a BMP assay it is essential to find out exactly what the characteristics of the substrate to be digested are.

Firstly, any uncertainty about the origin of the substrate tested should be avoided. Therefore, when dealing with plants, crops or other inhomogeneous materials, details on the part used for testing should be included. For example, the BMP tests of various components of *Jatropha curcus* ranging from 80 to 968 mL CH_4 g^{-1} VS_{added} [19]. Then, the description of the part used must be considered as a key parameter.

Secondly, the general characteristics of the substrate to be assayed should always be analyzed and the moisture, the total solids (TS) and the volatile solids (VS) should be quantified and controlled. It should be pointed out that some samples are problematic for TS and VS determination due to a possible loss of volatile organic matter during the drying process, including at low temperature or freeze-drying [21]. It is important to note that although specific methane yield on a VS basis is not a constant due to variations in organic matter composition, the VS content could be used as a primary indicator of the methane potential. It is noteworthy to mention that for energy crops and crop residues, the content and availability of VS which are able to produce methane is influenced by factors related to biomass production such as location, climate, variety, cultivation management and maturity stage at harvesting time [15,20,22,23].

Further information about the nature of VS can be assessed taking into account:

(i) Component composition. Not all VS are equal and therefore they exhibit different rates and extents of biodegradation during AD. The organic substance can be subdivided into: fats, proteins, carbohydrates and lignin. Proteins, lipids and extracted fractions of carbohydrates are usually the soluble parts, while the fibrous components represent the structural lignocellulosic content, in which case solubilization is very difficult. So, biodegradability is limited by the crystallinity of the cellulose and the lignin content [24].
(ii) Elemental composition. Another approach for characterisation involves the quantification of the content of certain elements (C, O, H, N and S). This information can be used to determine the empirical formula of the substrate.
(iii) Chemical oxygen demand (COD). This parameter is commonly used to characterize the total organic content of wastewater,

whereas it is not frequent for SOS. A simple explanation is that standardized methods are available for the measurements of COD for water and wastewater. However, COD measurements for solid substrates have been traditionally specifically adapted, where the samples have to be properly homogenized and diluted. Recently, good results were obtained using a modified method to measure the COD content of solid substrates without dilution [25]. In addition, it has been demonstrated that analytical performance in the measurement of COD of samples that are difficult to analyze, such as solid substrates and liquid samples with high suspended solid content, can be improved by regular participation in proficiency testing schemes [26]. In any case, COD is a very important analytical parameter because it is needed for modelling the energy balance of an anaerobic digester [27].

Further data on the composition of the SOS under test can be used to calculate theoretical methane yields by different approaches [28]. Although the theoretical potential provides only a basis for the quality of the substrate as a methane producer, some research estimated the methane yield without experimental work, based simply on its chemical composition [29]. However, the practical methane yield obtained in a reactor will always be lower than theoretical due to a number of factors [30]:

- Part of the organic material is often inaccessible due to binding of particles or structural organic matter.
- Some compounds are poorly degraded or not at all degraded anaerobically (e.g. lignin, peptidoglycan, etc.).
- A fraction of the substrate is used for cellular growth and maintenance. Although this portion may vary considerably depending on the operating conditions and substrates, in practice, 5–15% of COD removed can be considered typical as biomass cell factor [31,32].

2.1.2. Particle size

Particle size and the size reduction procedure may influence biodegradation results. It is generally accepted that hydrolysis is the rate-limiting step in anaerobic digestion of particulate substrates [33]. Surface area and particle size are important characteristics in determining the initial degradation rate. The size of the feedstocks should be limited, otherwise the digester may clog and it would also be difficult for microorganisms to carry out their digestion. In the case of substrates with low biodegradability, it is normally accepted that a size reduction of the particles and the resulting enlargement of the available specific surface can improve the biological process [34].

Little research has been carried out to determine the effect of particle size of solid substrates on methane yield [34–40]. The majority of results reported that methane yield was inversely proportional to particle size, but also some results reported no tangible effect on the kinetics of methane production. Since the relationship between particle size and biodegradability is not yet clarified, to allow for the results to be compared, the particle size should be comparable. A particle size of $\leq$10 mm is suggested. If the material used is difficult to reduce in size, it should be cut, broken or otherwise processed until the desirable size is achieved [41].

2.1.3. Concentration

One of the most important parameters for a batch assay design is the load of the solid substrate introduced into the digester. If the load is too low, although it limits the possibility of inhibitory effects, the microorganisms will exhibit a low metabolic activity and very low quantities of gas will be produced. If the load is too high, the biogas measurement may be more reliable but an overload situation in which intermediate volatile fatty acids (VFA) may build up, resulting in gas production inhibition.

Little detail about the influence of this parameter was found in the literature. Hansen et al. [42] described a laboratory procedure for the determination of BMP using 2% TS to more than 100 solid waste samples. On the other hand, the VDI 4630 guideline specified that the content of solids should not exceed 10% if an adequate mass transfer is to be assured [41].

2.2. Inoculum (INO)

Blok et al. [43] pointed out that even when the experimental conditions of batch test procedures can be harmonised, some variability in the results will always remain due to the biological nature of the test systems. The characteristics of microorganisms collected for use as inoculum can vary for the same treatment plant (daily or seasonal variations of flow-rate and substrate composition) and can be different from one treatment plant to another (operating conditions: organic loading rate, solid retention time, etc.).

The inoculum used for BMP assays must be fully characterized. Although subject to limitation, the easiest way to define the inoculum concentration is from the amount of volatile suspended solids (VSS). However, due to the inaccuracy of this determination in such samples, for the majority of anaerobic sludges VS are used as a measure of microorganism content. In any case, the information available for these analytical parameters is inadequate because it does not distinguish between microbial biomass and any other particulate organic material present in the reactor. This is especially evident in manures, where the inoculum VS content is mainly represented by recalcitrant lignocellulosic residues and not active microbial biomass, while in a granular sludge most of the VS consist of microbial cells [30]. Nor is it possible to determine if the microbial biomass is alive or dead.

The influence of the inoculum on the batch tests is mainly depending of six factors: origin/source, concentration, activity, pre-incubation, acclimation/adaptation and storage.

2.2.1. Origin/Source

The inoculum source relating to BMP tests is not uniform in the literature. Digested sludge from municipal wastewater treatment plants (MWTP), soil extracts, industrial treatment plants, rumen and animal manures have all been used. Although the use of an inoculum from such different sources may favour the environmental relevance of the tests, it is certainly not ideal for standardization [43]. On the other hand, the reproducibility of the assessment can be improved when a non-predetermined inoculum source is used [1].

Different sources could lead to different biodegradability results as a consequence of the different levels of microbial population. For a defined inoculum, the methane yield of an organic substrate is directly related to the extent of solubilization, while the degradation rate will depend on the slowest of the three steps of the anaerobic digestion process, namely hydrolysis (solubilization), acidogenesis and methanogenesis [39].

In general, digested sludge from a running biogas plant is used. The digested sludge from MWTP should offer the most suitable source of a diverse and active inoculum. This is preferable for the following reasons: (i) sewage treatment plants are found worldwide, (ii) although sewage treatment plants are different, they do have common features.

2.2.2. Concentration

Practical experience has demonstrated that the level of concentration of inoculum affects the rate of biodegradation. Normally, the higher the inoculum concentration, the faster the anaerobic conversion of the substrate, and the quicker the test will be completed.

Moreover, the concentration affects the duration of the lag period and the susceptibility of degradation due to inhibitory effects [4].

For some normalized biodegradability tests for micro-pollutants and the initial BMP procedure, the amount of inoculum used is generally expressed as a percentage of volume (10–80%). Using this unit system, the initial content of biomass is proportional to the VS content of the inoculum, whose value can range in manures and granular sludges from 2–3% to 10% VS, respectively [30]. Therefore, this criterion should be avoided because of its ambiguity. It is often more meaningful to express the concentration of inoculum in a batch assay in terms of VS.

To study the anaerobic biodegradability of micro-pollutants, a low inoculum concentration (1–3 g $TS \cdot L^{-1}$) was suggested because inoculum also contributes to gas formation which can blur the results if it is relatively high in comparison with the compound being tested [3]. On the other hand, in the case of complex SOS a small amount of inoculum can lead to an overload in the process with acidification and methane production inhibition [44]. The literature survey shows that a wide range of concentration has been used up to date. The lowest value (2.1 g $VS \cdot L^{-1}$) was reported by El-Mashad and Zhang [45], while the highest value (37.2 g $VS \cdot L^{-1}$) was stated by Rincón et al. [46]. The VDI 4630 guideline suggested using a range of between 15 and 20 g $VS \cdot L^{-1}$ from seeding sludge [41].

2.2.3. Activity

Inoculum activity is one of three types of batch assays commonly used. The influence of inoculum activity was extensively researched and the results obtained were reviewed by Rozzi and Remigi [1]. Interest is still evident and a recent study carried out by Souto et al. [47] was entirely dedicated to this topic.

Traditionally, activity has been limited to assessing specific methanogenic activity (SMA), but for a better identification of the quality of the inoculum used, it has been recently suggested by Angelidaki et al. [30] that activity of the different groups of microorganisms involved in the anaerobic process should be determined.

The use of different positive control substrates can be used for measuring activity and also for checking if the anaerobic biodegradation assays are performing well, for quality control purposes. These reference substrates should not ferment too quickly and should be completely biodegradable. As far as biodegradability is concerned, the experimental values should be close to the theoretical ones, because, as reported previously, only a limited percentage of substrate is not converted into biogas and utilised for cellular growth and maintenance. Partial biodegradation has on occasions been observed when positive control substrates have been tested. This could have been due to faulty experimental equipment or to inactive sludge. If the experimental equipment is shown not to be faulty, the safest course of action is to repeat the assay with fresh sludge [42]. Cellulose is the most frequent substrate used for measuring the adequate level of potential performance. However, the number of BMP research works where this substrate has been used is very low compared with the huge amount of articles on BMP assays.

Regarding to the influence of the inoculum activity into anaerobic biodegradability a few research works were reported [48,49]. It is noteworthy that Tait et al. [50] used an abiotic sludge control (inactivated inoculum) to evaluate the indigenous activities of some bedding (wheat straw and rice husks) from piggery housing.

2.2.4. Pre-incubation

Pre-incubation of sludge before feeding reduces the volume of gas produced in the blank controls and has been postulated as a mean of improving the precision with which net gas production can be measured. Recently, the use of a "degassed" inoculum has been suggested where 2–7 days of pre-digestion seemed to give an optimum decrease in background gas production with acceptable increases in both the lag and the total incubation periods [51].

The literature shows that most studies regarding this factor are for micro-pollutants. Pre-incubation has been widely recommended for testing the anaerobic biodegradability of these substrates, because in such cases it is difficult to clearly relate biogas evolution to degradation of the test compound or to distinguish the amount of biogas produced by the sludge itself [4]. On the other hand, a pre-incubation time of up to 3 weeks had no significant effect on the estimation of gas production [3].

2.2.5. Acclimation/Adaptation

The preculturing of the inoculum with a substrate leads to the induction of metabolic pathways for biodegradation, an increase of microorganism affinity for the compound and also an increase in the number of specific degraders. However, this idea of adaptation, although widely accepted by the scientific community, has not previously been reported for BMP tests, where the reported tests fit well with the philosophy of using not acclimated inocula.

2.2.6. Storage

For micro-pollutants, sludge storage had no significant effect on the extent of degradation, but the duration of lag times could be affected, and, therefore, substrates could be degraded more slowly [2]. The effect of storage on the batch biodegradability test for SOS is also scarce in the literature. Angelidaki et al. [30] suggested that fresh sludge should be used whenever possible.

2.3. Experimental conditions

2.3.1. Gas measurement systems (GMS)

Gasometric methods are the most frequently used for determining anaerobic biodegradability. In such methods, biogas/methane production can be quantified either manometrically by keeping the volume constant and measuring the pressure increase, or volumetrically by providing constant pressure conditions allowing measurement of the gas volume. Techniques for measuring the rate and volume of gas produced from anaerobic biodegradability assays include different systems such as lubricated syringes, volume displacement devices, manometers or pressure transducers, manometer assisted syringes, or low pressure flow meters. In addition, some automatic gas flow meters may be considered as mixed volumetric/manometric systems.

2.3.1.1. Volumetric methods (Vol). The first description of a volumetric measurement system for biogas production consisted in the displacement of the piston of a glass syringe with its needle being inserted into the reactor [7]. Alternatively, liquid displacement systems were proposed. In this case the biogas produced inside the reactor moved into a suitable external vessel which contained a barrier solution and displaced an equivalent volume of liquid. More recently, the Eudiometer unit was described as a more sophisticated apparatus which operated by a liquid displacement technique [52].

It is important to mention that precaution must be taken with the barrier solution used so as to avoid certain biogas components being lost. For the improvement of this measurement system, it is better to use an alkaline solution for washing the biogas, which means that the sole methane fraction can be measured directly [1,53]. Another option is to collect the biogas in a gas sampling bag with low permeability [54]. This system avoids the problem of adsorption during long periods of contact with the barrier solution, but it has the disadvantage of requiring a complementary gas meter for measuring the volume of gas collected.

2.3.1.2. Manometric methods (Man). In a manometric respirometer, the biogas produced is confined inside the bioreactor and hence generates proportional overpressure. An early manometric method was the Warburg respirometer [55]. Later, the method was improved by introducing the use of a pressure transducer to measure the gas production [56].

For this method, complementary biogas analyses are needed for calculating methane production. The major difficulty in accurately quantifying the overall gas production arises from the solubility of carbon dioxide in the digesting liquor as it is affected by pressure, pH, the ratio of headspace to liquid volume, temperature and the complex thermodynamic equilibrium established between carbon dioxide and the carbonates/bicarbonates of calcium and magnesium [4].

Recently a digital pressure transducer, called OxiTop® (WTW, Germany) and originally developed for biochemical oxygen demand (BOD) measurements, has been reported as useful for anaerobic biodegradability assays [57].

2.3.1.3. Gas chromatography (GC). Dolfing and Bloemen [58] determined the SMA of a sludge based on the GC analysis of the headspace of closed anaerobic vials. They sampled with a pressure lock syringe, which allows quantification independent of the pressure prevailing in the reactor. The volume of methane can be estimated based on the molar fraction of this gas in the headspace.

Hansen et al. [42] sampled only 10 mL of headspace gas during the full BMP test (0.2 mL every time), which represents less than 0.7% of the headspace volume, and the results were, thus, not significantly affected by the change of headspace pressure.

2.3.2. Operational conditions (OpC)

2.3.2.1. Physical operational conditions.

2.3.2.1.1. Volume. The total reactor volume used for batch tests is inversely related to the number of replicate samples that could be tested at the same time using a prefixed amount of sludge and substrate. The nature of the substrate can also influence the selection of the ideal volume, because the more homogeneous the material, the smaller the volume of reactor required to determine methane potential more accurately. The results of the extensive literature review showed that a wide range of different total volumes were utilised for anaerobic biodegradability batch assays, ranging from 0.1 to 120 L. However, the most common and useful volumes used for BMP assays are lower than 1 L.

2.3.2.1.2. Temperature. Although anaerobic biodegradation can take place within a wide range of temperatures, AD processes strongly depend on temperature. Depending upon the temperature at which the process is carried out, three temperature ranges can be differentiated: thermophilic (45–60 °C), mesophilic (20–45 °C), and psychrophilic (<20 °C) [59]. The main problem at the low temperature is the decrease in the microbial consortia activity.

The majority of data in the literature refers to experiments performed at mesophilic temperature, with only some at thermophilic temperature. The reason could be that the anaerobic digestion process is efficient enough at 35 °C and there is little to gain by increasing the operational temperature when increased costs are involved [11]. Taking into account the important influence of temperature, comparatively few studies have been carried out to relate its influence on biodegradation assays in batch mode using solid substrates [60–62].

2.3.2.1.3. Stirring. Agitation of digesters can be carried out in a number of ways: manual shaking, magnetic stirrers, orbital shaker, etc. The main factors affecting the mixing method are intensity and duration. The effect of mixing on the general performance of anaerobic digestion is contradictory. The continuous mixing of the content of the bioreactor favours contact between the substrate and the microorganisms as well as the release of biogas into the headspace, but it may also damage the structure of the flocs or granules, thereby worsening the close interaction between the different microbial populations within the agglomerate [1].

For micro-pollutants the stirring process is invariably essential to the rate of gas production, whereas it is independent of the extent of degradation [5]. On the other hand, the influence of mixing on the anaerobic biodegradability assays of SOS has never been reported in detail, although an optional device for mixing the reactors thoroughly may be useful in most cases.

2.3.2.1.4. Duration. The performance time of a batch assay can be related with the kinetics of the process. The main drawback of BMP testing is that it is very time-consuming [63]. A wide range of incubation time was reported in the literature. Owen et al. [7] advised the use of an incubation time of 30 days, which enables the complete degradation of organic substrates in most cases. Hansen et al. [42] increased the incubation time to 50 days to ensure maximum degradation of organic matter that has a lower rate of anaerobic biodegradability, although they reported that typically 80–90% of methane potential can be produced during the first 8–10 days. A high incubation time of 365 days was reported by Lopes et al. [64], 240 days by Rao et al. [65], and 155 days by Kaparaju et al. [66]. On the contrary, a shorter period of 7 days was reported in some batch tests [67,68].

2.3.2.2. Chemical operational conditions.

2.3.2.2.1. Headspace gas. Different gases have been reported in the literature to flush the reactor headspace: N_2, a mixture of N_2 and CO_2, He and air. The mixture of N_2 and CO_2 has been reported as the most commonly used gas within the headspace. Different ratios of both components (70–80% N_2 and 20–30% CO_2) can be found. The content of CO_2 is related to the buffering power of the system. No extensive research has been carried out to study the influence of CO_2 on anaerobic biodegradation in batch mode, but experimental results using only N_2 were similar when different substrates were selected [69].

More worthy of comment is the use of air as gas within the headspace. Oxygenation of the sample by exposure to air or sparging with oxygen reduces the biogas/methane production in proportion to the degree of oxygenation [70,71]. However, surprisingly the results were not different when air was used as headspace gas [69].

2.3.2.2.2. pH and alkalinity adjustment. pH is a measure of the acidity or alkalinity of the liquid content of the reactor. Most methanogenic microorganisms have an optimum pH of between 7 and 8, while the acid-forming bacteria often have a lower optimum pH [44]. If the pH of the waste to be tested is outside the optimal range, and if there is insufficient buffer capacity, the anaerobic process will be inhibited. Therefore, to avoid underestimating the methane potential, most batch tests are carried out at pH values ranging from 7.0 to 7.8. If the pH needs to be adjusted, a basic diluted solution such as NaOH or lime, or an acid solution such as HCl, could be used.

Alkalinity is the capacity to neutralize acids that provides resistance to significant rapid changes in pH. It is also known as "buffering capacity". It is the result of the presence of various compounds (mainly bicarbonate, carbonate and hydroxides). A value of 2500 mg $CaCO_3 \cdot L^{-1}$ is considered to be normal for sewage sludge. A more desirable range of 2500–5000 mg $CaCO_3 \cdot L^{-1}$ provides a higher buffering capacity for which a much larger increase in VFA can be accommodated with a minimum drop in pH [72].

The initial BMP test procedure suggested using an alkalinity of 2500 mg $CaCO_3 \cdot L^{-1}$. Later, most procedures reported for micro-pollutant biodegradation tests used the phosphate/biphosphate species as the sole source of alkalinity. Recently, Pabón [57] reported the inhibitory effect of the applied phosphate buffer to BMP tests.

2.3.2.2.3. Mineral medium (MM). It is well documented that all microbial-mediated processes require nutrients and trace elements (metals and vitamins) during organic biodegradation. In fact, eight inorganic nutrients: nitrogen, phosphorous, sulphur, potassium, magnesium, sodium, calcium, and iron were reported as necessary macronutrients in synthetic media [44]. In addition, some metals (chromium, cobalt, copper, manganese, molybdenum, nickel, selenium, vanadium and zinc), known as trace metals, are considered micronutrients, most of which are necessary as part of the active site of enzymes. Trace metals need to be dosed when added to the reactors so as to maintain microbial metabolism and growth [73]. The dose added must balance the requirements to support high activity, taking into consideration that above this concentration, trace metals become inhibitory or toxic [74].

Literature reports on the effect of mineral medium in batch tests are very inconsistent in this respect, because they vary from one to the other:

- For micro-pollutant biodegradation, different mineral media were compared for their effect on background gas production, lag times, and extent of degradation [2]. There was no significant effect on lag times with any of the media. However, the extent of degradation did vary.
- In a similar way, there is no general consensus on BMP tests as to whether these growth factors are readily available. A question that may arise is to what extent nutrients and trace elements are necessary depending on their content in the inoculum and the substrate used, being this aspect especially crucial when degrading mono-substrate. For instance, Pobeheim et al. [75] obtained different concentrations of macro- and micronutrients when various sludges from agricultural biogas plants were analyzed. On the other hand, some substrates were characterised before anaerobic biodegradability assays and found that they contained a balanced concentration of macro- and micronutrients necessary for anaerobic microorganisms [76,77].

It is important to note that if the mixture of inoculum-substrate lacks an important element, biodegradability could be severely affected. In this way, some research works demonstrated the positive effect of the addition of some nutrients and metals [75,78,79].

2.3.2.3. Inoculum to substrate ratio (ISR). Chudoba et al. [80] reported that one of the most important parameters in activated sludge batch testing is the initial substrate/microorganism ratio (S_o/Xo). However, the role of the influence of the ISR on anaerobic biodegradation tests is not clear. Theoretically, the methane yield should be independent of the ISR and only affect the kinetics of the process. But, experimental data demonstrated that the ISR can influence both the extent and the rate of the anaerobic biodegradation process. Unfortunately, many research works do not include the ISR used in the experimental design. It is sometimes possible to calculate the ISR with the information provided, but not when the data of the substrate and/or inoculum VS content are omitted.

Owen et al. [7] gave no detail of the ISR in their procedure, merely recommending a 20% volume of inoculum and a substrate concentration lower than 2 g COD·L^{-1}. Doing calculations, the ISR of the initial BMP procedure can be considered to be approximately 1 (VS basis). The first report dealing with the influence of ISR was published by Hashimoto [81]. He showed that the methane yield was drastically reduced at an ISR below 0.25 (VS basis) using wheat straw as substrate. The methane production rate was also found to increase as the ISR rose stepwise to 2, after which it remained relatively constant. Later, Chynoweth et al. [37] determined the effect of ISR on the biodegradation of cellulose. The extent values were similar, but the methane production rate was slightly higher for the highest ISR. In addition, imbalance was explained by the presence of higher concentrations of VFA in the assays with the lowest ISR. Consequently, they modified the ISR of the batch test to 2 (VS basis). The same conclusion about the clear influence of the ISR on anaerobic degradation was reported by other researchers using different substrates [49,62,64,68,82–84].

Finally, taking into account the potential amount of VFA produced and the possible ammonium generated, if proteinaceous matter is present, each substrate probably has the best ISR for performing the assay. However, for the harmonisation of the anaerobic biodegradation assays it is necessary to work at a high ISR value. Considering that an ISR $\geq$ 2 has never been reported as inhibitory, it could be used as the mandatory ratio for future standardized tests, as the VDI 4630 guideline suggested [41].

3. Conclusions

The BMP results compiled in this review demonstrated the lack of uniformity in the data reported, probably due to different inocula and experimental conditions utilised. BMP tests made in one laboratory should be consistent with those made elsewhere. It should be desirable that comparability are not very different with others making similar measurements. A dedicated IWA task group on anaerobic biodegradability, activity and inhibition (TG-ABAI) is working on this topic since 2002.

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Appendix A. Methane yields of solid organic substrates

Solid Organic Substrate (SOS)			Methane Yield	Reference
Name	Part	Size(mm)	(mL CH_4/g VS_{added})	
Alfalfa			210	[23]
Alfalfa	Silage		226	[23]
Apple	Fresh wastes		317	[89]
Azolla	Whole plant		132	[85]
Bagasse		< 2	77[c]	[67]
Bagasse		0.85–5		[112]
Bamboo			250[a]	[90]
Banana	Peeling		289	[89]
Banana			400[a]	[90]
Banana	Peels	2	243–322	[99]
Banana	Waste stem	10–20	81–196[a]	[107]
Banana	Peeling		374–409	[36]
Barley	Whole plant silage	20–40	375	[87]
Barley	Straw	50–100	229	[93]
Barley	Waste silage		222	[122]
Barley	Waste		20	[123]
Barley	Residue	10	271	[126]
Black locust			300	[139]
Braken			180	[57]
Bread-wholewheat			N.R.	[60]
Brewery	Grain		N.R.	[108]
Brewing draffs			385–400	[117]
Brinjal	Stalk	2	374	[99]
Brinjal	Whole fruit	2	396	[99]
Buckwheat			320	[57]
Cabbage			150[a]	[90]
Cabbage (fresh)				[91]
Cabbage	Leaves	2	309	[99]
Cabbage	Stem	2	291	[99]
Cabbage-white	Leaves		382	[143]
Cabbage-white	Leaves silage		343	[143]
Cabomba			155–160	[127]
Calotropis procera	Leaves		280	[117]
Candy-black			390	[66]
Cardboard			217	[105]
Carrot			310	[57]
Carrot	Peeling		388	[89]
Carrot	Leaves	2	241	[99]
Carrot	Petiole	2	309	[99]
Cassava	Pulp		370	[129]
Cattail			350	[129]
Cauliflower	Leaves	2	190	[99]
Cauliflower	Stem	2	331	[99]
Cauliflower	Leaves		352	[143]
Cauliflower	Leaves		341	[143]
Cellulose			404	[19]
Cellulose			370	[37]
Cellulose			379	[42]
Cellulose			345	[89]
Cellulose			356	[91]
Cellulose			419	[99]
Cellulose			356–375	[128]
Cellulose			367	[138]
Cellulose		100 mesh	373	[138]
Cellulose		100 mesh	390	[139]
Ceratopteris	Whole plant		204	[85]
Chocolate			370	[66]
Clover		< 20	140–210	[66]
Cocksfoot			325	[118]
Cocksfoot		10	308–382	[135]
Coconut	Fibres	0.85–5	N.R.	[112]
Comfrey	Tops		334	[143]
Comfrey	Tops		323	[143]
Confectionery	Raw material		320	[66]
Coriander	Leaves	2	325	[99]
Coriander	Stems	2	309	[99]
Coriander	Roots	2	283	[99]
Coriander	Whole plant	2	322	[99]
Corn stover			N.R.	[102]
Corn stover		30–60 mesh	360	[138]
Cotton	Stalks-wastes		62[a]	[78]

Solid Organic Substrate (SOS)			Methane Yield	Reference
Name	Part	Size(mm)	(mL CH_4/g VS_{added})	
Cotton	Seed hull-wastes		86[a]	[78]
Cotton	Oil cake-wastes		104[a]	[78]
Cotton	Stalks		145	[95]
Cotton	Residues		365	[126]
Crops-mixture	Silage	50	320–510	[130]
Cyperas	Whole plant	10	38	[85]
Dhub grass			205–228	[36]
Diapers			204	[105]
Faba bean	Straw		440	[131]
Fat-pork			900	[42]
Fish waste	Various		390	[120]
Food packaging			318–349	[128]
Food waste	Leachate		478	[114]
Food	Wastes		245–510	[62]
Food	Wastes		425–445	[77]
Food	Wastes		472	[91]
Food	Wastes	20 × 50	301[a]	[94]
Food	Wastes		525	[116]
Fruit and vegetable	Wastes		470	[134]
Garbage	Waste	10 × 10 × 5	395	[65]
Garden pea	Pods	2	390	[99]
Gelatine			100–150	[42]
Giant knotweed			170–270	[22]
Gliciridia	Leaves		165–180	[98]
Glucose			351	[42]
Glucose			335	[138]
Gracilaria spp.			280–400	[12]
Gracilaria tikvahiae		20–30	190–230	[100]
Grape	Stalk		116	[93]
Grape	Marc		98	[93]
Grape	Pressings	2	283	[99]
Grape	Peduncle	2	180	[99]
Grass			267	[23]
Grass			374	[23]
Grass			N.R.	[60]
Grass			388	[89]
Grass			128–144[a]	[94]
Grass			320	[134]
Grass cuttings			300	[22]
Grass hay			270–350	[66]
Grassland			128–392	[15]
Green pea	Shells	10–20	194–220[a]	[106]
Green wastes			206–357	[62]
Grey	waste		147	[105]
Hydrilla	Whole plant		81	[85]
Ipomea fistulosa	Leaves		413–429	[36]
Jatropha curcus	Leaf lamina		227	[19]
Jatropha curcus	Leaf petiole		335	[19]
Jatropha curcus	Leaf entire		224–237	[19]
Jatropha curcus	Green fruit		326	[19]
Jatropha curcus	Yellow fruit		518	[19]
Jatropha curcus	Brown fruit		469	[19]
Jatropha curcus	Fruit hull		306	[19]
Jatropha curcus	Seed testa		80	[19]
Jatropha curcus	Seed kernel		968	[19]
Jatropha curcus	Seed entire		610	[19]
Jatropha curcus	De-oiled cake		230	[19]
Jerusalem artichoke			360–370	[22]
Jerusalem artichoke	Tops		309	[143]
Jerusalem artichoke	Tops silage		301	[143]
Kitchen waste			432	[122]
Kitchen waste		1–3	370–430	[124]
Kitchen waste			450	[134]
Ladies finger	Stalk		350	[99]
Laminaria		0.8	260–280	[37]
Leather fleshing			490	[136]
Lemon	Pressings		473	[99]
Lettuce	Residues		294	[89]
Lucerne	Whole plant silage	20–40	357	[87]
Lupine			310–360	[22]
Lupine (white)		< 0.2	260	[57]
Lupine (yellow)		< 0.2	260	[57]
Macrocystis		0.8	390–410	[37]
Maize	Mixture	0.5–3	268–366	[14]
Maize			398	[15]
Maize	Whole plant		282–419	[18]

Solid Organic Substrate (SOS)			Methane Yield	Reference
Name	Part	Size(mm)	(mL CH_4/g VS_{added})	
Maize		2–4	251–349	[20]
Maize			315	[23]
Maize	Silage		364	[23]
Maize	Bran		64 [(3)]	[67]
Maize			250–340	[74]
Maize		2	196–233	[83]
Maize	Whole plant silage	20–40	345	[87]
Maize	Fresh whole plant	10	300–400	[88]
Maize	Whole plant silage	various	370–410	[88]
Maize	Residues		317	[93]
Maize	Stalks		229	[95]
Maize	Residues	10	363	[126]
Maize	Whole plant		378	[140]
Maize	Whole plant silage		328–418	[140]
Mandarin	Peels	2	486	[99]
Mandarin	Pressings	2	433	[99]
Mandarin	Whole rotten fruit	2	494	[99]
Mandarin	Seeds	2	732	[99]
Mango	Peels	2	370–523	[99]
Marrow kale			310–320	[22]
Meadow foxtail			310	[118]
Meat and bone meal			351–381	[142]
Meat-cooked			482	[91]
Microcystis			94–141	[84]
Millet	Bran		590	[117]
Millet	Straw		390	[117]
Mirabilis	Leaves		241	[137]
Mirabilis	Leaves		327–341	[36]
Mustard	Tops		300	[143]
Mustard	Tops silage		326	[143]
Napiergrass		0.8	190–340	[37]
Napiergrass	Lamina	2	372	[99]
Napiergrass	Sheat	2	342	[99]
Napiergrass		< 20 mesh	288	[138]
Nettle			210–420	[22]
Newspaper		shredded	92	[138]
Newsprints			58	[105]
Oat		< 20	250–260	[66]
Oat			320	[22]
OFMSW			298–573	[28]
OFMSW		0.8	200–220	[37]
OFMSW		2–50	160–250	[40]
OFMSW			495	[42]
OFMSW			353	[45]
OFMSW			230–550[d]	[64]
OFMSW			92[a]	[94]
OFMSW			60–530	[96]
OFMSW			187	[97]
OFMSW		Screw press	450	[101]
OFMSW		Disc screen	450	[101]
OFMSW		Shredding	450	[101]
OFMSW		10	157	[103]
OFMSW			50–200[a]	[111]
OFMSW			186–222	[128]
OFMSW			360	[136]
Onion	Exterior peel	2	400	[99]
Orange	Peeling		N.R.	[60]
Orange	Peeling		297	[89]
Orange			115[a]	[90]
Orange	Peel	2	455	[99]
Orange	Pressings	2	502	[99]
Orange	Waste	< 7	490	[109]
Palm Oil	Fruit bunches		370	[129]
Paper			300[a]	[90]
Paper (coated)			84[a]	[94]
Paper (newsprint)			74[a]	[94]
Paper (office)			217[a]	[94]
Paper (bag)			250	[42]
Paper (office printer)			340	[105]
Paper			84–369	[128]
Paper and cardboard			109–128	[132]
Parthenium			140–152	[82]
Pea-green	Shell	10–20	194–220[a]	[106]
Pig waste			230–620	[61]
Pineapple	Peel	2	357	[99]
Pineapple	Leafy shoot	2	355	[99]
Pineapple	Peel		400	[129]

Solid Organic Substrate (SOS)			Methane Yield	Reference
Name	Part	Size(mm)	(mL CH_4/g VS_{added})	
Pomegranate	Peels	2	312	[99]
Pomegranate	Rotten pulpy seeds	2	430	[99]
Pomegranate	Whole rotten fruit	2	342	[99]
Pomegranate	Pressings	2	420	[99]
Poplar (Populus sp)		0.8	230–320	[37]
Poplar (Populus sp)			350–420	[139]
Potato	Waste		320[c]	[54]
Potato			390	[89]
Potato	Peel	2	267	[99]
Potato	Pulp		N.R.	[108]
Potato	Pulp	3–10	332	[113]
Potato	Peel-pulp	3–10	377	[113]
Potato	Fruit water		323	[113]
Poultry slaughterhouse	Waste		550–670	[133]
Quinoa			330	[57]
Radish	Shoots	2	293–304	[99]
Rape	Straw		240	[22]
Rape	Oil seed		800–900	[42]
Rape			290	[57]
Rape	Straw		420	[131]
Rape	Tops		334	[143]
Red clover			280–300	[22]
Reed canary grass			340–430	[22]
Reed canary grass		10	253–351	[135]
Rhubarb			320–490	[22]
Rhubarb	Tops		316	[143]
Rhubarb	Tops silage		345	[143]
Rice-boil			294	[91]
Rice	Straw		347–367	[36]
Rice	Straw		347–367	[36]
Rice	Straw	50–100	195	[93]
Rice	Straw		215	[95]
Rice	Straw		270–290	[115]
Rice	Straw		340	[129]
Rosebay willow			200	[57]
Rye-winter			140–275	[15]
Rye-winter	Straw	< 2	360	[131]
Ryegrass			360	[118]
Saccharum spp.			270–310	[92]
Salvinia	Whole plant		242	[85]
Salvinia			50	[127]
Sapota	Peels	2	244	[99]
Sapota	Whole rotten fruit	2	327	[99]
Sargassum spp.			150–180	[12]
Sargassum		0.8	260–390	[37]
Scirpas	Whole plant		66	[85]
Seaweed		2–3	90–120	[125]
Sisal fibre waste		2–100	176–216	[34]
Sisal pulp			320	[120]
Sisal pulp waste	Leaf tissues + fibres		120–240	[121]
Sludge-kraft pulp mill			90[b]	[141]
Sludge-sulfite pulp mill			320[b]	[141]
Sorghum		0.8	260–390	[37]
Sorghum	Whole plant silage	20–40	362	[87]
Sorghum	Lamina	2	367	[99]
Sorghum	Sheath	2	407	[99]
Sorghum	Inflorescence + flowers	2	480	[99]
Sorghum	Inflorescence + grains	2	538	[99]
Sorghum	Roots	2	228	[99]
Sorghum		0.8	280–400	[104]
Spartina			290	[57]
Starch			348	[42]
Sugar beet			340	[22]
Sugar beet	Leaves	2	231	[99]
Sugar beet	Pulp		N.R.	[108]
Sugar beet	Pulp	3–5	430	[113]
Sugar beet	Tail	1–3	481	[113]
Sugar beet	Tops		360	[143]
Sugar beet	Tops silage		381	[143]
Sugarcane			230–300	[37]
Sugarcane	Residue	1	177	[126]
Sunflower			428–454	[15]
Sunflower	De-oiled cake	< 2	107–227	[68]
Sunflower	Whole plant silage	20–40	345	[87]
Sweet clover			290	[57]
Sweet gum			260	[139]
Sweet pea			370	[57]

Solid Organic Substrate (SOS)			Methane Yield	Reference
Name	Part	Size(mm)	(mL CH_4/g VS_{added})	
Switch grass			191–309	[119]
Sycamore			380	[139]
Tall fescue		10	296–394	[135]
Tea	Residue	10	67	[126]
Teak			270[a]	[90]
Textiles			228	[105]
Timothy		10	308–365	[135]
Timothy-clover grass			370–380	[22]
Tomato	Skins and seeds		218	[93]
Tomato	Whole rotten fruit	2	211–384	[99]
Triticale			212–286	[15]
Triticale			290	[57]
Turnip	Leaves	2	314	[99]
Ulva spp.			94–177	[13]
Ulva spp.		20–30	220–330	[100]
Utricularia	Whole plant		132	[85]
Vetch			290	[57]
Vetch–oat mixture			400–410	[22]
Water hyacinth		0.8	190–320	[37]
Water hyacinth	Whole plant	1.6–12.7	130–180	[38]
Water hyacinth			244	[95]
Water hyacinth			60–190	[127]
Water hyacinth			350	[129]
Wheat	Straw	0.088–6	227–249	[36]
Wheat	Straw	10	299–331	[81]
Wheat	Straw		267	[86]
Wheat	Straw silage		396	[86]
Wheat	Whole plant silage	< 1	276	[87]
Wheat	Straw	<1	297	[110]
Wheat	Straw	30–60 mesh	302	[138]
Wheat	Straw	<30 mesh	333	[138]
Wheat-winter			229–343	[15]
Wheat-winter		5–15	311–360	[46]
White fir		<40 mesh	42	[138]
Willow (Salix spp.)			130–300	[37]
Willow (Salix spp.)		<0.8	280–370	[139]
Winter bean			350	[57]
Winter harley			300	[57]
Wood grass		<20 mesh	291	[138]
Yard	Wastes		345	[116]
Yard	Wastes		123–209	[128]

[a] mL CH_4/ g TS_{added}.
[b] mL biogas/g VS_{added}.
[c] mL CH_4/g $VS_{removed}$.
[d] mL biogas/g $VS_{removed}$.
N.R.–not reported.

Appendix B. Description of experimental BMP procedures

Reference	INO			GMS	Physical-OpC							Chemical-OpC			ISR
	Source	VS	C_o		Capacity (L)		Temp		Mixing		TD	Gas	Adj	MM	
		(%)			TV	WV	°C	System	Type	Times	(days)		pH/Alk		VS basis
[12]	MWTP		10 (%-vol)	Vol (syringe)	0.282	0.100	35				60	N_2-CO_2 (70–30%)		Yes	1
[13]	No inoculum				30		35				64				
[14]	Energy crops	58		Vol (liq-disp)	1		38	TWB	Cont (mag bar)	10 s/10 min	45				2 (TS)
[15]				Vol (liq-disp)	1		38	TWB							
[18]	Digested material			Vol (gas meter)	20		37				50				
[19]	Manure + Veg wastes		20 (%-vol)	Vol (syringe)	0.135	0.075	35				105	N_2-CO_2 (70–30%)		Yes	2
[20]				Vol (liq-disp)	0.5	0.4	35	TWB			35	N_2			
[22]	Cow manure + Byproducts	79	13.3 (g VS/L)	Vol (liq-disp)	2	1.5	35		Batch (manually)	1/day	≈150	N_2	Yes ($NaHCO_3$)		
[23]				Vol (bag + meter)	2	1.5	35	TC			35				
[28]			20 (%-vol)	GC	2		55				50				
[34]	Sisal WW sludge	48		Vol (syringe)	1	0.6	33	Ambient room	Batch (manually)	2/day	65	N_2			0.35
[36]	Manure (cattle)			Vol (liq-disp)	5	4	37		Batch (mag bar)	2 min/3h	56		Yes [$Ca(OH)_2$]		
[37]	MWTP		20 (%-vol)		0.250	0.100	35				46	N_2-CO_2 (70–30%)		Yes	2
[38]	Various			Vol (gas meter)		55	35	TC			60		Yes ($NaHCO_3$)		
[40]	MSW-leach bed			Vol (gas meter)	220	110	38	TC	Mixer	290 rpm	20–40		Yes ($NaHCO_3$)	Yes	
[42]	Manure + Org wastes		400 (mL)	GC	2	0.5	55	TC	Batch (manually)	Ocassionally	50	N_2-CO_2 (80–20%)			2 (%-w/vol)
[45]	OFMSW	59	2.1 (g VS/L)	Man	1	0.500	35		Batch (manually)	1/day	30	He			1
[46]	MWTP	65	37.2 (g VS/L)	Vol (liq-disp)		1.5	35	TWB	Cont (stirrer)	300 rpm	96			Yes	2
[54]	MWTP	57		Vol (bag+meter)	0.5	0.3	37	TWB	Cont (shaker)	70 rpm	50	N_2-CO_2 (80–20%)			0.15–5.4
[57]	MWTP + distillery			Man (Oxytop®)	1	0.600	35	TC	Batch (manually)		40	N_2	Yes ($NaHCO_3$)	Yes	2
[60]	Paper-mill WW				1	0.600	20–40		Cont (shaker)	100 rpm	55	N_2-CO_2 (70–30%)	Yes ($NaHCO_3$)	Yes	1.4–2.1
[61]	Manure (digested)		60 (%-vol)			0.5/2	55				30–40	N_2-CO_2 (80–20%)		Yes	
[62]	MWTP meso/thermo	5652		Man	1	0.600	35 50		Batch (manually)	1/day	25	He			0.3 0.2–0.6
[64]	Rumen (bovine)				20						365				0–0.17
[65]	Manure (cattle)		15 (%-vol)	Vol (liq-disp)	3.25	2	26		Batch (manually)	1/day	240				
[66]	Manure (cow)	63	11.3 (g VS/L)		2.0	1.5	35				155	N_2-CO_2 (80–20%)			0.3–0.7
[67]	Rumen (sheep)				0.125	0. 050	39		Cont (shaker)	100 rpm	7	N_2		Yes	
[68]	Brewery (UASB)	75	15 (g VS/L)	Vol (liq-disp)	0.300	0.250	35	TWB	Cont (stirrer)	40 rpm	7	N_2	Yes ($NaHCO_3$)	Yes	0.5–3
[75]	Maize silage		15 (g VS/L)	Vol (liq-disp)	2	1	35		Batch (mag bar)	8 × 15 s/day	30			Yes	1.5
[77]	MWTP	51		Vol (liq-disp)	1	0.500	50		Batch (manually)	1/day	28	He			0.4–0.6

Reference	INO			GMS	Physical-OpC							Chemical-OpC			ISR
	Source	VS	C_o		Capacity (L)		Temp		Mixing		TD	Gas	Adj	MM	
		(%)			TV	WV	°C	System	Type	Times	(days)		pH/Alk		VS basis
[78]	MWTP			Vol (liq-disp)	0.250	0.100	35	TC			23	N_2-CO_2 (75–25%)	Yes ($NaHCO_3$)	Yes	
[81]	Manure (cattle)	60	10–90 (%-vol)	Vol (syringe)	0.119	0.050	35	TC			150	N_2			0.03–11
[82]	Manure (cattle)				2		26		Cont (mag bar)		35	N_2			
[83]	MWTP	63	15 (g VS/L)	Vol (liq-disp)		5	35	TWB	Cont (stirrer)		20	N_2	Yes ($NaHCO_3$)	Yes	1–3
[84]	Manure (cattle)			Man	0.250	0.120	35	TWB	Batch (manually)	2/day	30	N_2-CO_2 (80–20%)			0.5–2
[85]					1		37	TC			35				
[86]				Vol (liq-disp)	1		38				42				
[87]				Vol (liq-disp)	0.250		37								3 (TS)
[88]	Manure (cow)			GC	2.140	0.590	55					N_2			
[89]	Waste mixtures			Vol (liq-disp)		3.5	55	TWB			15–22	N_2-CO_2 (75–25%)		Yes	1.3–2
[90]	OFMSW		99 (%-w)		0.135	0.050	30	TC			45	N_2			
[91]	MWTP		20 (%-vol)				37	TC			28				
[92]	Manure (cattle) dung						35				100				2
[93]	Mixture (codigestion)			Vol (bag+meter)	2		40		Batch (manually)	2/day	40				2
[94]	OFMSW		30 (%-vol)	Vol (bag+meter)	2	0.8	40						Yes	Yes	
[95]	Manure (cattle) slurry			Vol (liq-disp)	2.5		35	TC			120				
[96]	Various		25 (%-w)		1.1		55	TWB			60				
[97]	Waste mixture				0.5		37		Cont (shaker)					Yes	
[98]	Manure (cattle)			Vol (syringe)	3		32		Cont (mag bar)		30				
[99]	Vegetable wastes		20 (%-vol)	Vol (syringe)	0.135	0.075	35				100	N_2-CO_2 (70–30%)		Yes	2
[100]	Seaweeds				2	1.7	32		Batch (manually)	15 sec/day	58				
[101]	Manure + Org wastes						55				50				
[102]	Rumen (goat)			Vol (liq-disp)	0.250	0.100	25–40	TC	Cont (shaker)	130 rpm	10	N_2	Yes ($NaHCO_3$)	Yes	0.3–0.8
[103]	MWTP			Vol (liq-disp)	1	0.9	30	WB			919	N_2		Yes	
[104]							35				60				
[105]	MWTP	53		Vol (bag+meter)	2	1.6	35				237	N_2-CO_2 (80–20%)	Yes ($NaHCO_3$)	Yes	
[106]	Manure (cattle)			Vol (liq-disp)	0.300	0.275	40				25–35	N_2	Yes		
[107]	Manure (cattle)			Vol (liq-disp)	0.300	0.275	40				57	N_2	Yes (NaOH)		
[108]	Effluent two-stage	60 85		Vol (gas meter)	5		35		Cont (mag bar)		37–85				0.8–2
[109]	MSW	53	10 (g VS/L)		0.120	0.060	55				122	N_2-CO_2 (80–20%)	Yes ($NaHCO_3$)		3.2
[110]	Manure (cow)			GC	0.118	0.040	55		Static		60	N_2			
[111]	MWTP		10 (%-vol)	Vol (syringe)	0.250	0.100	35				45			Yes	

Reference	INO			GMS	Physical-OpC							Chemical-OpC			ISR
	Source	VS	C_o		Capacity (L)		Temp		Mixing		TD	Gas	Adj	MM	
		(%)			TV	WV	°C	System	Type	Times	(days)		pH/Alk		VS basis
[112]	Rumen										30				
[113]	Vegetable+ Crops			Vol (liq-disp)	1		37.5	WB	Batch (mag bar)	10/30 min	28–38				3 (TS)
[114]	MWTP		20 (g VS/L)		0.500	0.250	35		Cont (shaker)	100 rpm	28	N_2	Yes ($NaHCO_3$)		10
[115]	Rice	60	3.3 (g VS/L)	Vol (liq-disp)	5	4	22		Cont (mag bar)		120	N_2	Yes (NaOH)		
[116]	Pig manure + Food waste				0.100	0.060	55				28		Yes ($NaHCO_3$)		
[117]	Manure (cattle)		8–10 (g VS/L)	Vol (syringe)	0.100	0.050	35	TWB	Batch (manually)	2/day	70	N_2	Yes (NaOH)		0.5
[118]	Digested material			Vol (gas meter)		2	35	TWB			28				
[119]	Manure (swine)			Vol (gas meter)	30	20	35	TC	recirculation	3/day (1 min)	50–60				
[120]	Sisal WW sludge	52		Vol (syringe)	1	0.6	27		Batch (manually)	2/day	25–29	N_2			0.4–20
[121]	Activated sludge	55	4.9 (g VS/L)	Vol (syringe)	0.5	0.3	37	TWB	Cont (shaker)	70 rpm	32–85	N_2-CO_2 (80–20%)	Yes ($NaHCO_3$)		0.65
[122]	Brewery (UASB)			Man	0.160		37			150 rpm	100		Yes ($NaHCO_3$)		0.43 (TS)
[123]	Brewery (UASB)			Man	0.160 120	80	37			150 rpm			Yes		0.14
[124]	Brewery (UASB)	65		Man	0.160		37			150 rpm			Yes ($NaHCO_3$)	Yes	0.74
[125]	MWTP			Vol (bag + meter)	0.5	0.3	37	TWB	Cont (shaker)	70 rpm	30	N_2-CO_2 (80–20%)	Yes ($NaHCO_3$)		1–2
[126]	Cow manure + Paper mill			Man	1	0.2	30		Shaker		30	N_2	Yes (phosphate)	Yes	1.5
[127]	Primary WAS + foodwaste			Man	0.200	0.100	38		Stirring	Sampling				Yes	
[128]	Primary WAS		20 (%-vol)	GC	0.275	0.100	35	TC			60	N_2-CO_2 (70–30%)		Yes	3–4
[129]	Manure (pig)			Vol (liq-disp)	0.120	0.065	37				90	N_2-CO_2 (70–30%)		Yes	3 (?)
[130]	Manure (cow)	77		Vol (liq-disp)	1	0.750					70–80	N_2	Yes ($NaHCO_3$)		1–2
[131]	Manure	69		GC	0.100	0.025	42	TWB	Cont (shaker)	100 rpm	67	N_2			2 (?)
[132]	MWTP			Man	1.130	0.800	35				100	N_2		Yes	0.03 0.04
[133]	MWTP	64	40–80 (%-vol)		0.118	0.050	35		Cont (shaker)		27	N_2-CO_2 (80–20%)			1.1–4.3
[134]	MWTP			Man	1.140										1.81
[135]	Manure, silage and byproducts	79	19 (g VS/L)	Vol (bag + meter)	1	0.750	35		Batch (manually)	Sampling	95	N_2	Yes ($NaHCO_3$)		1
[136]				Vol (liq-disp)	0.500	0.400	35		Batch (stirrer)	15/30 m 140 rpm	40			Yes	
[137]	Manure (cattle)	89	50 (%-vol)		1		36	TC	Batch	2/day	56				
[138]	MWTP		10 (%-vol)	Vol (syringe)	0.260	0.130	35		Cont (mechanical)	15 rpm	60–133	N_2-CO_2 (70–30%)		Yes	

Reference	INO			GMS	Physical-OpC						TD	Chemical-OpC			ISR
	Source	VS	C_o		Capacity (L)		Temp		Mixing			Gas	Adj	MM	
		(%)			TV	WV	°C	System	Type	Times	(days)		pH/Alk		VS basis
[139]	MWTP		20 (%-vol)	Man	0.250	0.100	35	TC			60–100	N_2-CO_2 (70–30%)	Yes ($NaHCO_3$)	Yes	2
[140]	Landfill leachate			Vol (liq-disp)	2.5	1.5	35		Batch (manually)	1/day	21	N_2			
[141]	Paper-mill WW			Man	0.160	0.100	35	TWB	Cont (shaker)	200 rpm	35	$N_2/CO_2/H_2$ (80-10-10%)		Yes	
[142]	MWTP		3.3 (g VS/L)	Vol (liq-disp)	1.175	0.600	35	TC	Batch (manually)	2/day	10–72	N_2	Yes (HCl/NaOH)		
[143]	Plant material			Vol (liq-disp)	3		35					25–36	Yes (NH_4OH)		

Source: MWTP-municipal wastewater treatment plant; WW: wastewater; VS: Volatile solids; C_o: Concentration (g VS/L); (%-w): % in weight; (%-vol): % in volume; GMS: gas measurement system; Vol: volumetric system; (liq-disp): liquid displacement; Man: manometric system; GC;gas chromatography; TV: Total volume; WV: working volume; Temp: Temperature; TWB: thermostatic water bath; TC: thermostatic chamber; Mixing: Cont-continuously; mag bar: magnetic bar; TD: Test duration; Adj: Adjustment of pH and/or alkalinity; MM: Mineral medium; (?): Units were not reported.

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